Extracellular Vesicles From the Cells Harboring HIV Defective Provirus Initiate Inflammatory Cascade

380

Hongyan Sui[1], John G. Bernbaum[2], Hiromi Imamichi[3], H. Clifford Lane[3], and Tomozumi Imamichi[1]

1 Laboratory of Human Retrovirology and Immunoinformatics, Frederick National Laboratory, Frederick, MD 21702; 2 Integrated Research Facility, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Frederick, MD 21702; 3 Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

sponsored by the National Cancer Institute

BACKGROUND

It has been demonstrated that over 95% of provirus present in the peripheral blood of people living with HIV (PLWH) on combination antiretroviral therapy are defective. Defective viruses have long been considered biologically irrelevant, as they cannot encode infectious viruses. However, recent studies have shown that these defective viruses can be translationally active. Their biological impact remains unclear. Therefore, there is a need for a better understanding of the biological function of defective HIV proviruses.

METHODS

Cell lines harboring defective proviruses derived from H9MN were used in this study (Fig. 1). The H9-FI harbored an intact full-length HIV-1 provirus, the H9-FD contained a full-length defective provirus with a 1-bp inserted frameshift, and the H9-SD possessed a 2.4-kb internal deletion. Cytopathic effect was determined using H9-FD or H9-SD cells co-culturing with MT-2 cells. Exosomes (EXOs) were isolated from cell culture supernatants of H9-FD or H9-SD using multiple steps of ultracentrifugation (Fig. 2). Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analysis were used to characterize the EXOs. A CodePlex secretum proteomics workflow was used to measure multiple cytokine inductions.

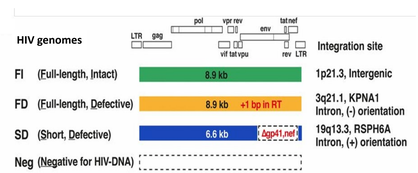


Fig. 1 Genomic information for the H9 provirus clones used in the study (*H. Imamichi et al., 2020*).

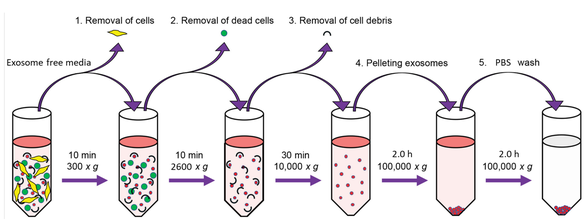
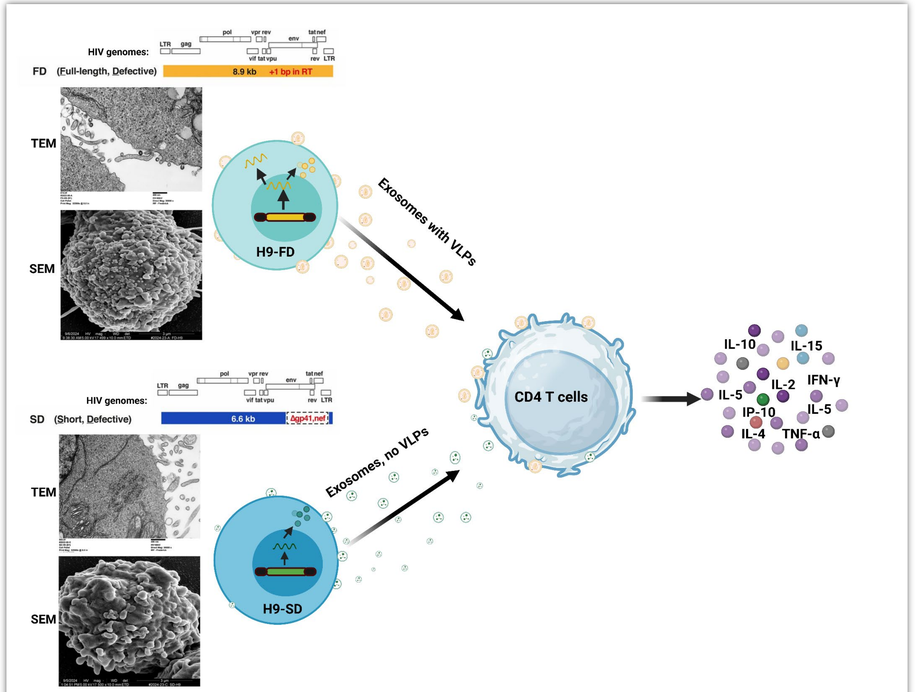


Fig. 2 The diagram of Exosome isolation from the supernatants (*Adapted from https://bio-protocol.org/e3576*).



- **The cells harboring defective HIV provirus produced HIV virus-like particles, 90-95% of these were "immature" particles.**
- **A mixture of exosomes produced by cells harboring defective HIV provirus induced the production of multiple cytokines, including IFN-γ, IL-10, IL-15, IL-17A, IL-2, IL-4, IL-5, IP-10, sCD137, and TNF-α, in human primary CD4+ T cells.**

Created by BioRender.com

PERSPECTIVES

HIV-1 defective proviruses are not silent and may play a critical role in stimulating innate immune responses and may be associated with persisting immune activation in PLWH despite successful anti-retroviral therapy.

REFERENCES

H. Imamichi, M. Smith, J.W. Adelsberger, T. Izumi, F. Scrimieri, B.T. Sherman, C.A. Rehm, T. Imamichi, A. Pau, M. Catalfamo, A.S. Fauci, H.C. Lane, Defective HIV-1 proviruses produce viral proteins, Proc. Natl. Acad. Sci. U.S.A.117 (7) 3704-3710, https://doi.org/10.1073/pnas.1917876117 (2020).

CONTACTS

Dr. Hongyan Sui suih@mail.nih.gov

Dr. Tomozumi Imamichi timamichi@mail.nih.gov

This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. 75N91019D00024. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute

Frederick National Laboratory is a Federally Funded Research and Development Center operated by Leidos Biomedical Research, Inc., for the National Cancer Institute

RESULTS

➢ Cells harboring defective proviruses induced syncytium formation in MT-2 cells.

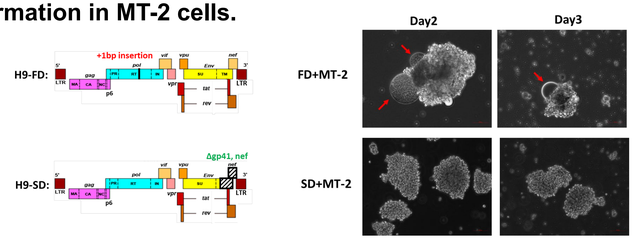
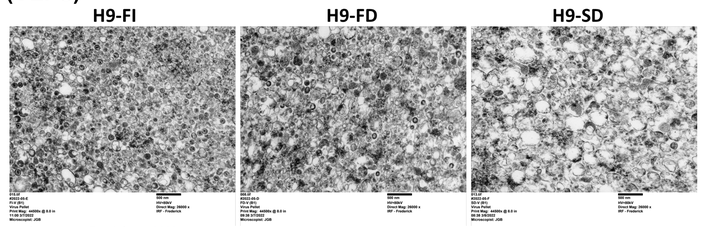


Fig.3 Defective HIV provirus cell clones were cocultured with MT-2 cells with 1:1 ratio. The cells were observed under microscopy at day 2 or day 3 after the co-culturing. Brightfield Images were captured with 10X magnification.

➢ Defective HIV provirus cells produced virus-like particles (VLPs)



- Immature Particles: 10%
- Mature Particles: 90%
- Immature Particles: 90-95%
- Mature Particles: 5-10%
- No any unequivocal lentivirus particles was observed.

Fig.4 TEM analysis of exosomes secreted from T cell clones harboring defective HIV proviruses. Exosomes were isolated from the supernatants of H9-FI (positive control), H9-FD and H9-SD cell cultures. Exosome pellets were prepared and fixed for thin-section TEM analysis. Morphological forms of the lentivirus particles were analyzed, and the frequency of each form was quantified. The scale bar denoted 500 nm.

➢ A mixture of exosomes from the cells harboring defective HIV proviruses induced multiple inflammatory cytokines in human primary CD4+ T cells.

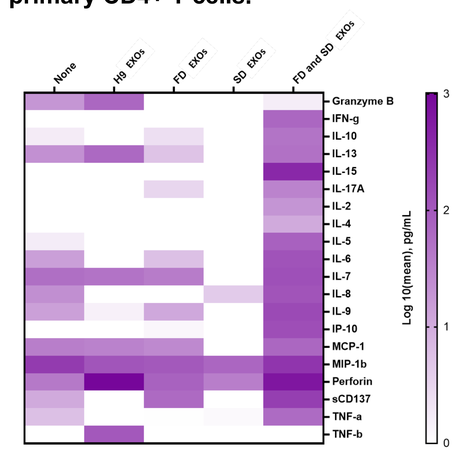


Fig.5 Human primary CD4+ T cells were stimulated by exosomes of H9, H9-FD, H9-SD or a mixture of exosomes from H9-FD and H9-SD, at 10 particles/cell. The cell supernatants were harvested at 24 h after stimulations. The supernatants were then subjected to multiplex cytokine assay using the IsoPlexis Human Adaptive Immune Panel Kit for detecting IFN-γ IL-10, IL-13, IL-17A, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MIP-1β, perforin, sCD137, TNF-α, and TNF-β. The results (log10 pg/mL) were displayed by means of duplicate detections. White boxes indicated no cytokine inductions. The results are representative of two independent experiments.